The Development of the Ovary and Ovarian Egg of a Mosquito, A n o p h e l e s m a c u l i - p e n n i s, Meig.

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With Plates 17–20.

Contents.

INTRODUCTION.

THE examination of mosquito ovaries was first commenced with the idea of finding out at what period the ovaries of the hibernating females commence to develop, so that an accurate knowledge of the time at which the mosquito lays the first eggs of the season might be determined. From an examination of sections of the ovaries, it soon became evident that the oocyte nucleus behaved in a somewhat unusual manner during the period of yolk formation. I therefore decided to examine this in detail and at the same time observe what might be termed the grosser anatomy of the developing ovary and oocyte. An immense amount of work has been done on the oogenesis of insects, but most of this has been confined to the detailed examination of the complicated nuclear changes which take place during this period. The mosquito, however, is peculiarly unsuitable for the study of the differentiation of the oocyte and of the prophases of maturation which takes place in the end chamber. As this is very small, in order to examine some of the stages, it would be necessary to cut very thin sections of ovaries containing oocytes with large yolk-masses and in some cases chorion as well. This is an operation which I found quite impossible to perform. The finer structure of the oocyte nucleus has therefore only been studied where it is rendered necessary in order to give a connected account of the development of the oocyte.

To the best of my knowledge the only references to the development of the ovarian egg of the mosquito are contained in two short papers by Christophers. In one of these (2) he gives a very general description of the ovary and egg-follicles, while in the other (3) he describes the development of the egg-follicle from the examination of fresh material. As the information in both these papers is of a very general nature it has been found necessary to repeat portions of it, as otherwise a connected account of the development of the ovary could not be given.

I will take the opportunity here of expressing my deep

MATERIAL AND METHODS.

The majority of the mosquitoes were taken during the latter part of their hibernating period and the first few weeks after they had regained their activity and had commenced to feed normally.

In order to eliminate the possibility of being misled by artefacts due to fixation, the following method was employed. Each batch of material was divided into three different parts ; two of these were fixed in different re-agents, and in the case of the third the ovaries were dissected out in salt solution and one ovary of each insect was rapidly transferred to one fixative and the other to another. In this way the effect of different fixatives on ovaries in the same stage of development could easily be compared.

In the cases where the ovaries were not dissected out, the abdomen alone was fixed, and this was slit along each side with a fine needle in order to allow the easy entrance of the fixative.

It was found that in the case of the less-developed ovaries much the best results were obtained with those which were dissected out, but far less distortion was produced in more mature ovaries fixed while still in the abdomen. This was probably due to the fact that the surrounding tissues only allowed the fixative to reach the ovaries gradually and so prevented rapid osmosis.

A number of different fixatives were used, the principal of which were Flemming, both with and without acetic, Petrunke-

witsch, Zenker, and alcoholic Bouin. Of these, Petrunkewitsch was by far the most useful for general purposes as its penetration is very good, a most important consideration when dealing with oocytes containing a large yolk-mass, particularly when the egg-walls are present. For the finer cytological details Flemming with acetic gave the best results, though Flemming without acetic appeared to give a more perfect fixation, but the latter had the disadvantage that the chromatin did not stain as distinctly as it did with unmodified Flemming.

Another fixative of which I made considerable use was the modified Bouin described by Sheppard (**27**). This I used in conjunction with the method of staining described by the same author, i. e. bulk staining with carmalum and counter-staining with Grübler's light green. Using this method the fixation was excellent, and the double staining gave very beautiful preparations—yolk and chorion staining bright green and the protoplasmic structures red. This property was very useful in following the branching nucleus through the yolk-mass and in following the production of chorion by the epithelial cells. The fixative had the disadvantage, however, of making the material brittle.

The stain principally used was Grenacher's haematoxylin counter-stained with dilute Lichtgrün picric. For the latter the ordinary Lichtgrün picric solution (0·2 grm. Lichtgrün dissolved in 100 c.c. of a saturated solution of picric acid in absolute alcohol) was diluted with about ten times the bulk of 90 per cent. alcohol. The counter-staining was done under observation, as Lichtgrün appears to displace the haematoxylin and the reaction requires to be stopped when all the yolk has become green and the protoplasmic structures are still blue. Using this method the branching nucleus can easily be followed amongst the yolk granules.

Heidenhain's iron haematoxylin counter-stained with eosin or orange G was also extensively used and was particularly useful for the finer nuclear details, but as it stained the yolk-mass dense black it was not satisfactory for the more developed oocytes.

Most of the material was embedded in paraffin in the ordinary way, but this was not very satisfactory, as oocytes containing large yolk-masses broke up easily and it was difficult to cut uninterrupted series. Towards the end of my work I obtained much more satisfactory results using the double-embedding collodion and paraffin method described by Newth (**21**). Using this method uninterrupted series of thin sections were easily obtained.

Hibernating Mosquitoes and First Period of Egg Development.

During the winter, female A. maculipennis may be found in cowsheds, church towers, and in fact in almost any dry and comparatively warm place. They pass the winter in a semidormant state, but they are found to feed a little during this period, as occasionally an insect with a little blood in the abdomen may be observed. A microscopic examination of an insect at this stage shows that the fat bodies are relatively very large, the ovaries are always very small, in the ' resting stage ', and the spermatheca is full of sperms. In cases where the first batch of eggs had already been laid and the second was developing, the spermatheca was seen to contain sperms, though they were not in such a compact mass as in the hibernating insects. As the first males of the season had not emerged at this period, it would appear that one fertilization of an insect is sufficient for more than one period of oviposition.

The period at which the ovaries of mosquitoes first commence to develop depends on the warmth of the season and also on the locality. Thus in 1919 the majority of the insects taken at the end of March in Kent showed considerable development of the ovaries, while a similar degree of development of the ovaries was not found till about three weeks later in the Shrewsbury district. On March 23, 1920, however, insects with the ovaries in the resting stage were only found with great difficulty in the Shrewsbury district. This is no doubt due to the early spell of fine weather in that year.

Warm weather acts merely as a stimulus to the activity of
the insects and causes them to go out and seek food. The
stimulus which gives rise to egg development appears to be
a good meal of blood. Numerous experiments have been
carried out to determine whether blood is necessary for the
production of eggs in mosquitoes. To the best of my knowledge
in only one case have mosquitoes been induced to lay when
fed on any substance other than blood. S. K. Sen (**26**) suc-
ceeded in inducing Stegomyia scutellaris to oviposit
by feeding with milk or peptone sweetened with cane-sugar,
and in two instances was successful when the insect had fed
on nothing but cane-sugar. I carried out a number of feeding
experiments on A. maculipennis, feeding them on sugar
and water, with and without the addition of peptone, and on
dates, bananas, and other fruit, all of which the mosquitoes
consumed very greedily, but in no case did any development of
eggs take place. In all my sections of abdomens in which the
eggs are developing, the gut is found to contain blood, with
the exception of the final stage, in which the eggs are fully
developed, when the gut is always empty. As all these speci-
mens were collected in cowsheds, this does not prove that blood
is always necessary for the production of eggs, but it appears
to me certain that this normally is the case.

In hibernating mosquitoes the abdomen is very narrow and
flattened dorso-ventrally, but when they take their first meal
of blood in the spring the abdomen becomes almost globular
and distended to its limits with blood. In an insect which has
recently fed, the abdomen shows a large semi-transparent
uniform mass of blood, while a small whitish mass is seen
through the cuticle at the anal extremity. This consists prin-
cipally of Malpighian tubules but also contains the ovary.
On the second day the posterior portion of the blood is very
dark red and opaque, while the remainder is as before. In
sections the dark-red portion is seen to consist of partially-
digested blood containing very distorted corpuscles, while the
remainder appears to be quite fresh and might easily be
mistaken for a fresh meal of blood. The white mass at the

anal extremity has enlarged somewhat owing to the growth
of the ovary. By the third or fourth day the blood-mass
is seen to be much reduced, and the whitish mass, the ovaries,
about half fills the abdomen. The blood-mass is reduced to
a mere spot or is entirely absent by the sixth or seventh day,
and, if the weather is warm enough, the eggs are then laid
during the night. In cold weather, however, the insects may
wait several days before oviposition.

These observations were made on a number of insects
collected in a calf-pen. At the time of collection their abdo-
mens contained semi-transparent blood-masses and they had
only recently fed. They were kept in jars in the laboratory
and the eggs were laid though they received no further food.

The period elapsing between the time of feeding and ovi-
position appears to be about a week, which agrees very closely
with Christophers's observations on A. rossi in India (3), in
which case the period is given as six days.

I only succeeded in observing insects during the process of
laying in two cases, and in both the eggs were laid within an
hour of darkness setting in. The insects floated on the water
by spreading their long legs over it and frequently dipped their
proboscides into the water. When disturbed they flew off the
water with ease and seemed in no danger of drowning. The
actual oviposition I was unable to observe as the mosquitoes
refused to lay in the light.

Female Genital Organs.

In the ' resting stage ' the genital organs of the adult female
mosquito consist of two small ovaries lying ventro-laterally
in the posterior portion of the abdomen. Each of these com-
municates posteriorly with an ovarian tube, and the two ovarian
tubes unite to form a common duct, the gynaecophoric canal,
which opens to the exterior at the posterior end of the eighth
segment. A spermatheca, consisting of a thick perforated
chitinous shell and surrounded by a layer of large clear cells,
gives off a very thick-walled sperm-duct to the gynaecophoric

canal, which it enters a short distance anterior to the genital
aperture. A mucous gland, which consists of very large goblet
cells, also communicates with the gynaecophoric canal, close
to the entrance of the sperm-duct (fig. 7).

The ovary is surrounded by two sheaths, an outer bag-like
structure, the investing membrane, and an inner membrane,
which is closely applied to the egg-follicles and fits them like
a glove : the fingers of the glove are the follicular tubes and the
portion joining up the fingers encloses the lumen of the ovary.
The investing membrane passes anteriorly into a tubular
suspensory filament, which is fixed to the hypodermis at the
junction of the fourth and fifth segments, in a dorso-lateral
position. This filament is very long in the young ovary,
but it becomes quite short when the ovary is fully developed.

The two sheaths are identical in structure, and consist of
a structureless membrane, over one surface of which large
nuclei are found. From these radiating muscle-bands pass
over the membrane. These nuclei and muscle-bands are on
the inside of the investing membrane and on the outside of
the follicular tubes, and muscle-bands pass from the nuclei
of the one to the other, thus traversing the cavity between
the two sheaths and linking the investing membrane and the
follicular tubes together. so forming a very complicated
muscular system (fig. 24).

The muscle-bands of the sheaths are striped in the normal
manner, thus differing from those of most insect ovaries (see
J. Gross, **9**). They form broad bands close to the point of
origin from the nuclei and taper away from here and branch,
some of the finer branches appearing to consist of only a few,
or even a single muscle-fibre, as the ' striations ' consist of
bead-like, deeply-staining nodes on a fine thread (fig. 24).

It would probably be more correct, in many cases, to consider
that the nuclei are placed at intervals on the muscle-fibres,
rather than that they are the origin of the fibres. From an
examination of fig. 28 it will be seen that many muscle-bands
pass through the cytoplasm of the cells, and merely become
slightly indefinite there. The ' striations ', though somewhat

distorted, are still placed at regular intervals. In other cases, however, the nuclei certainly appear to be the origin of the muscle-bands.

Over the greater portion of the surface of the investing membrane the muscle-bands radiate in the normal manner (fig. 28), but towards the junction with the oviduct they gradually become reduced to two laterally-placed bands which pass transversely to the long axis of the ovary (fig. 29). Finally, the investing membrane passes over the oviduct and the muscle-bands now form the circular muscles of the oviduct. In a similar manner the muscle-bands of the follicular tube membrane pass insensibly into the longitudinal muscles of the oviduct, inside which is found a layer of columnar cells surrounding the lumen of the oviduct.

If an ovary of a living insect is dissected out in salt solution, a vigorous rhythmatic peristaltic movement is noticed. This may be produced by the stimulus of the salt solution, but there is little doubt that this movement takes place in the living insect, at least when the eggs are being laid. The movement is undoubtedly due to the muscular system described, and the basket-work arrangement of the muscle-fibres is ideal for compressing the ovary and so pressing the eggs into the oviduct. The muscle-bands which pass from the investing membrane to the follicular tubes are probably of use in drawing the latter off the eggs, a process which takes place some time before the eggs are laid.

A number of very characteristic cells are found in the space between the two sheaths, and also between the follicular tubes and the egg-follicles ; one or more is almost always to be found in the region of each terminal chamber, between it and the follicular tube membrane (fig. 25). These cells consist of large nuclei embedded in a mass of very much vacuolated protoplasm, from which fibres are frequently seen to pass. The exact nature of these cells I have not been able to determine, but I am of the opinion that they have some relation to the tracheal system. The fibres seen passing from them are probably tracheal endings, but they are so fine that it is difficult to

determine their nature and they might equally well be proto-
plasmic strands. In several cases, however, I have succeeded
in tracing some of these fibres to the bundles of tracheal endings,
so that some at least are tracheal in nature.

It is possible that these vacuolated cells may be leucocytes
as they agree in structure and size with Vaney's (**31**) descrip-
tion and illustrations of the leucocytes in the larva of G a s t r o -
p h i l u s   e q u i , but the fact that fibres enter them throws
considerable doubt on this theory.

The tracheal system in the ovaries is very highly developed.
Tracheae from the fourth and fifth segments go to the ovaries
and branches of these penetrate the investing membrane.
These tracheal trunks branch repeatedly in the space between
the two ovarian sheaths. The final branches consist of exceed-
ingly fine tubes, in which no spiral filament can be distinguished.
These pass to the various parts of the ovary in bundles, the
tubes being joined together by the tracheal cells which occur
at intervals along the bundles. When such tracheal cells are
cut transversely they appear to be very much vacuolated,
owing to the numerous tubes passing through the cytoplasm.
The individual tubes eventually become free from the bundles
and end in the tissues of the ovary.

A moderately large tracheal branch passes into the base of
each follicular tube and gives rise to numerous bundles of
tracheal endings. In the young ovary these have a very
characteristic appearance, and are seen as a prominent coiled
mass at the base of each follicular tube. This allows for
expansion when the ovarian follicles increase in size. The
ultimate endings of these tubes are difficult to discover, but
I have noted some entering cells of the ovarian follicles and
isolated tracheal endings may be seen in almost any part of
the follicular tubes.

Inside the follicular tube is an egg-string consisting of an end
chamber followed by two or three egg-follicles in various stages
of development. These follicles are joined together by cellular
stalks consisting of a single row of cells (fig. 26). The last
' stalk ' or funicle runs from the posterior and most-developed

follicle to the portion of the follicular tube membrane which invests the lumen of the ovary, with which it fuses.

The whole of the egg-string is invested by a thin structureless membrane, the tunica propria, which may also be regarded as the basement membrane of the follicular epithelium. I am here using the term ' tunica propria ' in the sense defined by J. Gross (**9**). The term has been used by many authors as synonymous with ' peritoneal membrane ', a practice which has led to much confusion. The peritoneal membrane is represented in the mosquito by the two ovarian sheaths.

Normally the tunica propria can scarcely be observed, as it is very closely applied to the follicular epithelium : but it frequently happens that the follicles degenerate, and then the tunica propria can easily be seen as a somewhat wrinkled, structureless bag surrounding the remnants of the follicle (fig. 24).

Each follicle consists of an oocyte and seven nurse-cells completely surrounded by a single layer of cubical cells, the follicular epithelium.

GENERAL LINES OF DEVELOPMENT OF OVARY AND EGGS.

Before giving a detailed description of the various changes which take place during the oogenesis of A . m a c u l i p e n n i s , I will first give a general outline of the development of the egg-follicles and of egg formation, as a comprehensive view of the whole subject will render it more easy to follow the detailed descriptions of the different processes which together produce the mature egg, but which, for sake of clearness, have to be dealt with separately. Also a description of the anatomy of the mature egg will be given, as with a knowledge of this it will be possible to understand the object of the various processes.

The earliest stages of oogenesis are to be found in the end chamber. This consists of a central mass containing comparatively large nuclei, which often vary considerably in appearance but are not definitely divided into nurse-cells and oocytes, and of a peripheral layer containing smaller nuclei which give rise to the follicular epithelium.

At intervals a mass of cells is cut off from the end chamber
and consists of seven nurse cells and an oocyte surrounded by
a follicular epithelium. This follicle increases in size till it
reaches the resting stage (fig. 26), which is characteristic of
the ovaries of hibernating females. When the most-developed
follicles of the ovary are at this stage the ovary is very small
and quite transparent.

If the ovary of an insect which has just had a meal of blood
be examined in a fresh condition, it will be found that a white
opaque cloud is visible surrounding the nucleus of the oocyte.
This consists of fine yolk. In living ovaries at a slightly later
period it will be found that the whole oocyte is opaque white
and occupies about half the follicle. In sections this opaque
mass is found to consist of both coarse and fine yolk, and the
oocyte nucleus is no longer spherical but sends out blunt
processes into the yolk (fig. 18).

At a still later stage the follicles are elongated instead of
almost spherical, and are quite opaque except for a small
transparent cap, consisting of nurse-cells, and a thin investing
layer of follicular epithelium. The nucleus has now become
very much branched, branches passing throughout the yolk-
mass and appearing to be in some connexion with the nurse-
cells, which are evidently in a state of activity. A new structure
has now appeared between the follicular epithelium and the
yolk-mass. This consists either of globules or of a layer of
gelatinous material, and is the commencement of the inner wall.

Shortly after this stage the nurse-cell nuclei are extruded
from the yolk-mass and come to lie in the follicular epithelium,
forming a cap over the anterior end of the egg. The oocyte
nucleus has now reached its maximum condition of branching
and shortly afterwards breaks down. The inner wall is
thick but still gelatinous. The follicular epithelium becomes
modified in two lateral areas and gives rise to the floats. The
rest of the epithelium secretes the chorion over the whole
surface of the egg, that portion which contains the extruded
nurse-cell nuclei giving rise to the micropyle apparatus.

Finally, the follicular epithelium degenerates into a mere

membrane surrounding the fully-formed eggs ; these lie in the lumen of the ovary, as the follicular tubes have contracted and merely cover the less-developed follicles and a small portion of the anterior end of the fully-formed eggs. The eggs, however, still lie in the position in which they developed (fig. 8).

When the eggs are being deposited they appear to break through the remains of the follicular epithelium and then pass down the oviduct to the exterior, the sperms entering the micropyle immediately before the eggs are laid.

ANATOMY OF THE MATURE EGG.

The mature egg is more or less cigar-shaped and is provided at each side with a float (fig. 9). It is, however, noticeably thicker at one end than the other, and I consider the thick end as anterior, as it is anterior in the ovary. The portion of the egg which is uppermost when floating I shall refer to as the dorsal surface.

The egg can be divided into three main parts—the outer wall or chorion, the inner wall, and the yolk-mass.

The outer wall of the egg of A. maculipennis appears to be identical in nature to that of other insect eggs, that is, it is formed of chorion, which closely resembles chitin, but differs from it in that it is soluble in warm KOH solution, whereas chitin may be boiled in concentrated caustic potash for hours without effect.

The structure of the chorion of the mosquito egg shows a high degree of specialization. It consists essentially of a thin envelope surrounding the whole egg, two floats placed dorso-laterally, and a very beautifully-formed micropyle apparatus situated immediately below the extreme anterior end of the egg.

The envelope is completely covered with processes of four kinds. The ventral surface is thickly covered with short knob-like processes (fig. 41), and some of these are slightly larger than the remainder and are so arranged that they divide the whole of the ventral surface of the egg into polygonal areas (fig. 9). These areas probably have some connexion with the form of the epithelial cells, but they appear to be too large to be produced by individual cells.

The dorsal surface and the portion of the envelope lying under the floats are covered with very different processes. They are longer, and thin sheets of chorion radiate from a central axis, so that in section the processes are star-shaped. At the top of each process a cap-like structure joins all the radiating thin sheets together (fig. 41).

At the line of division between the dorsal and ventral types of processes there is a single row of much longer processes which extend as a band from the terminations of the floats to the tips of the egg (fig. 9).

The fourth type of process only occurs in small numbers and is found at each end of the egg. This type is a comparatively large boss-like structure consisting of a solid mass of chorion, and seven or eight are found at the extreme anterior and posterior ends of the egg (fig. 40).

The floats consist of a single sheet of chorion attached to the chorion envelope along its ventro-lateral surface only. The sheet curves round till it almost touches the dorso-lateral surface so enclosing a considerable cavity. The whole of the chorion sheet which forms the float is highly corrugated (fig. 9).

The whole of these structures, with the possible exception of the ' bosses ', appear to serve the purpose of supporting the egg on the surface of the water. The ventral processes enclose a film of air, which cannot be expelled by the water owing to its surface tension and the closeness of the processes to one another. The floats enclose a relatively large volume of air, and again surface tension prevents the entrance of water. The band of long processes, from the floats to the tips of the egg, probably helps to support the egg by making use of surface tension directly, i. e. by lying on the surface film of water. The comparatively long dorsal processes do not help to support the egg normally, but if an egg is sunk it will be found that the relatively thick film of air enclosed by these always causes the egg to regain the surface with its dorsal surface uppermost.

If a drowning mosquito lays its eggs under the water it is

found that they all sink, so it is obvious that the buoyancy of the eggs is entirely due to the entrapped air.

The micropyle apparatus consists of a very thin disk-like membrane surrounded by a thick supporting ring. The central portion of the membrane is produced into a funnel, which passes through the inner wall to the interior of the egg, and the cavity of the funnel is the micropyle (fig. 41).

The supporting ring is somewhat irregular on the outer side, but the inner edge is very regularly scalloped, and the top portion of the ring in each scallop is produced towards the micropyle so that it overhangs the rest and together with the disk forms a shallow pocket (figs. 40 and 41).

Radiating out from the region of the micropyle to the point of junction of each ' scallop ' is a very fine ridge. These are thickenings of the disk corresponding to the divisions of the cells which give rise to the apparatus. These ridges, together with the ' scallops ', mark the apparatus off into well-defined areas. There are normally eight of these areas, but I have found examples of the apparatus with from seven to ten.

The funnel continues right through the inner wall and ends at the inner edge of the latter. It does not, however, communicate direct with the cytoplasm of the egg, but is sealed up by a small globular portion of the inner wall which for convenience I shall term the stopper (fig. 41).

A consideration of the structure of the micropyle apparatus and of the genital aperture leads me to the following theory as to the function of the former.

While the egg is passing through the gynaecophoric canal it is no doubt considerably compressed by the muscular walls of this canal. This would cause the thin membranous disk of the micropyle apparatus to be forced outwards, and it would probably lie level with the top of the supporting ring. By the time the micropyle apparatus has reached the region where the spermathecal duct opens into the gynaecophoric canal, the bulk of the egg has left the genital aperture, and thus the pressure on the egg is released. The membranous disk is now able to resume its original position, and in so doing would probably

draw sperms into the saucer-shaped cavity of the apparatus.
Here the sperms would be directed by the radiating ridges to
the micropyle, and would then pass down the funnel and
between the stopper and the inner wall to the protoplasm of
the egg.

When an egg is freshly laid on water it is nearly white,
but after a few hours it becomes grey, and by morning it is
usually, if not always, quite black. The whole of this change
of coloration is due to the inner wall, which is transparent
when laid but later becomes opaque black. In several cases
insects in captivity have laid their eggs on dry media instead of
on water, and in none of these cases did the eggs become black :
they merely turned dirty yellow. It would thus appear that
water has something to do with the production of the dark colora-
tion, though how the water gets to the inner wall is not clear.

Besides changing colour the inner wall changes in character
after the deposition of the egg. If a freshly-laid egg is placed in
strong acid or alkali rapid expansion of the inner wall takes
place, and it is seen first to become rapidly wrinkled and finally
to burst through the chorion with explosive force.

An egg which has become black, treated in the same way does
not appear to be acted upon. Also if a freshly-laid egg is
crushed under a cover-slip the inner wall is seen to be gelatinous,
and oil-like globules may be broken off if a little pressure is
applied. If treated with osmic acid these globules become
brown, so that there may be a chemical, as well as a physical,
resemblance between the inner wall and oil. If an egg which
has become black is crushed it is found that the inner wall is
no longer gelatinous, but is hard and somewhat brittle as it
cracks with pressure.

The yolk-mass occupies the whole of the egg inside the inner
wall. It consists of an alveolar protoplasmic mass in the
vacuoles of which yolk granules of two kinds are found (fig. 11).

The more obvious form of yolk consists of comparatively
large granules 0·003 mm. to 0·01 mm. in diameter, which are
proteid in character, as the following reactions show.

If treated with copper sulphate solution followed by excess of

caustic potash, i. e. the ' Biuret Reaction ', the granules turn a beautiful violet colour.

Nitric acid turns the previously white yolk light yellow, and when excess of ammonium hydroxide is added the yolk turns a brilliant orange colour. (Xanthoproteic Reaction.)

With osmic acid the granules turn yellow or yellow brown.

These granules appear to be homogeneous and solid, as they can be broken by the pressure of a cover-slip.

The other type of yolk consists of small granules 0·001 mm. in diameter, and these are found surrounding the granules of coarse yolk (fig. 11). They are chemically quite different from the large granules as they do not respond to any of the above reactions. They are certainly not fat globules, as might be expected, as they are not coloured in any way by osmic acid, either alone or in the presence of chromic acid in the form of Flemming without acetic : a test described by Gatenby (7). I have not succeeded in making the fine yolk granules respond to any chemical reaction or stain in any way. In sections they appear as clear vacuoles, but they must be more than mere drops of watery fluid, as, if an egg is broken in water, they are scattered through the liquid as minute spheres which exhibit a very pronounced dancing movement. This movement comes to rest after a few hours, so that it is probably due to diffusion currents from the granules.

It may be noted here that the fine yolk is a definite constituent of the mature mosquito egg, and is not an inter-mediate substance produced during the formation of yolk, as in the case of the ' granules adipeux ' of Pholcus phalan-gioides according to Van Bambeke (1).

The protoplasmic portion is very inconspicuous in the mature mosquito egg. In sections it is seen as a network of fine threads, the meshes of which are occupied by the yolk granules (fig. 11). At the periphery the protoplasmic threads are slightly thicker than in the remainder of the egg, and they form an ill-defined cortical layer. This layer is thickest at the two extremities of the egg, in each of which it forms a small area of granular protoplasm free from yolk. Occasionally a few

disconnected fragments of the branching nucleus can be distinguished in the yolk-mass, even in eggs which have been laid.

The only other protoplasmic structure visible is a small mass of granular protoplasm situated about a quarter of the length of the egg from the anterior pole and in the centre of the yolk-mass. This appears to be the remains of the chromatin residue, but before the egg is laid no nuclear structure can be distinguished in it. A short time after oviposition, however, this mass is found to contain minute chromosomes.

DIFFERENTIATION OF GERM-CELLS AND THE FIRST PERIOD OF GROWTH OF THE EGG-FOLLICLES.

The growth of the egg-follicle falls naturally into two periods. The first period is from the time when the follicle is separated off from the end chamber up to the formation of the ' resting stage '. Growth is arrested at this point and only recommences after the insect has had a good meal of blood, when the follicle enters upon the second period of growth, which culminates in the formation of the mature egg. In the second and later generations of eggs, however, the two periods run concurrently, i. e. while the primary follicles are undergoing the second period of growth the secondary follicles are undergoing the first, and when the former have formed the mature egg the latter have reached the ' resting stage '.

If an end chamber in an early stage of development be examined it will be found to consist of a central mass containing comparatively large nuclei surrounded by a layer in which smaller nuclei are found scattered somewhat irregularly. Cell divisions cannot be distinguished and the mass is probably a syncytium (fig. 24). The larger central nuclei are those of the oogonia and oocytes, and the smaller peripheral ones give rise to the follicular epithelium.

The nuclei of the oogonia vary considerably in appearance even in the same mass, but in the earlier stages of the end chamber I have been unable definitely to separate the true oocyte from the nurse-cells. Occasionally mitotic figures are found in the central mass (fig. 31). This is no doubt the stage

at which the oogonia divide to produce the oocytes, all but one of which become modified to form nurse-cells.

In many end chambers, where the foregoing process has no doubt already taken place, the proximal nucleus is quite distinct from the remainder. It is clearer and contains a well-defined spireme, while the other cells are somewhat darker, and, though they often also appear to contain a spireme, this is never so sharply defined and usually can only be made out with difficulty. This proximal nucleus is the true oocyte nucleus, while the cells lying above it are the nurse-cells, and at the distal end of the chamber the remaining oogonia are found.

The epithelial layer grows between the mass of nurse-cells and the oogonia, so that the former, together with the oocyte, are completely enclosed in a follicle. The fact that there are seven nurse-cells and one oocyte suggests that they are produced by three successive divisions of a single oogonium, seven of the daughter cells becoming nurse-cells and the eighth the true oocyte. Occasionally an aberrant number of cells are included inside the follicular epithelium : eight large cells, and at the distal end a number of smaller cells, apparently eight in number. In this case the epithelial layer has surrounded two masses of daughter cells instead of one. I have only found such follicles in young ovaries, so that the mass of smaller cells evidently does not take part in the development of the follicle and probably degenerates. This further supports the theory that the nurse-cells and oocyte are the daughter cells of a single oogonium.

When the follicles are first formed the follicular epithelium consists of a comparatively small number of cells. These multiply rapidly by mitotic division (fig. 32), and at this stage no clear cell divisions are visible. Also there is only a small quantity of cytoplasm, the epithelium consisting principally of a large number of closely-packed nuclei. This mitotic division takes place throughout the first period of growth till, when the resting stage is reached, the full number of epithelial cells is attained and also the nuclei have reached their full size. During the second period of growth the epithelium increases

very considerably in area, but I am convinced that this is entirely due to the increase in size of the individual cells. In no case have I seen any sign of mitotic division during this period. Further, counts were made of the epithelial cells in median longitudinal, and transverse sections of follicles in various stages of development, and the average number of cells visible in a section was found to be practically constant irrespective of the size of the follicle.

The ' funicle ' arises by the local proliferation of the epithelial cells of the septum between the end chamber and the young follicle. Shortly after the young follicle is cut off from the end chamber a number of nuclei are found closely packed one above the other in the form of a short rod. This rod-like structure is found in one side of the septum, and this asymmetrical position is retained throughout the growth of the follicle, as will be readily understood when it is considered that the micropyle apparatus is formed immediately under the ' funicle ', and this is not terminal but ventral in position.

The nuclei in this rod-like structure continue to divide, forming a long string. The rest of the septum splits and gives rise on the one hand to the follicular epithelium and on the other to the epithelial layer of the end chamber. The only portion of the septum which does not split is that which contains the rod-like series of nuclei, and this now forms the funicle.

When the egg-follicle is first formed the nurse-cell nuclei often contain a more or less indefinite spireme and there is very little cytoplasm. As the follicle grows the nuclear contents become arranged in much convoluted bands which appear to be directly derived from the spireme. These bands are somewhat peculiar in structure. They consist of a non-staining ribbon of linin, across which lie a large number of chromatin bands giving an appearance somewhat resembling that of a striped muscle (fig. 30). These convoluted bands lie round the periphery of the nucleus with the result that individual sections give a very wrong idea of the appearance of the nucleus. This is due to the fact that the nurse-cell nuclei are gigantic (0·02–0·03 mm.) in diameter, so that each nucleus is

cut into a number of sections and the idea of the continuity of the convoluted bands is lost.

Towards the centre of the nucleus a large irregular nucleolus is found imbedded in a mass of linin. This is joined to the convoluted bands by linin threads. The nuclear membrane is very thick and is always plainly visible.

When the follicle has reached the resting stage each nurse-cell nucleus is found to be surrounded by a large mass of cytoplasm which is limited by a definite cell-membrane. The cytoplasm is slightly granular and takes up rather more stain than that of the younger cells.

The earliest stages of the oocyte nucleus which I have been able to distinguish contain a number of deeply-staining chromatin loops all of which arise together from one side of the nucleus (fig. 12). This is evidently the ' bouquet stage ' of the prophases. Usually a small nucleolus is also seen, and in the few cases I have observed in which this is not visible it is possible that it was hidden by the chromatin threads. At this early stage the nucleolus is a spherical vesicle and only stains very lightly.

As the nucleus grows the nucleolus becomes relatively larger, takes up stain rather more readily, and soon several vacuoles become visible in it. While this is taking place the chromatin threads wind themselves round the nucleolus and invest it tightly (fig. 13). The nucleolus continues to grow at a more rapid rate than the rest of the nucleus, while the chromatin strands do not appear to grow at all. The result is that the enlarging nucleolus gradually pushes the investing chromatin strands off itself, and these are then seen as a small mass of closely-woven threads at one side of the nucleolus (fig. 16). These threads concentrate into a closely-packed mass in which the individual chromatin threads can no longer be distinguished, and the whole has the appearance of a small dark nucleolus at the side of the true nucleolus. For want of a better term I shall refer to this mass as the ' chromatin residue '. This is embedded in a mass of linin which also invests the nucleolus and thus holds the two closely together (fig. 17). This arrangement

persists until after the oocyte has left the resting stage. Frequently, however, the chromatin residue is very difficult to find in oocytes in the resting stage. Close examination reveals the fact that it is not only very closely applied to the surface of the nucleolus, but is situated in a shallow depression in the latter and thus does not disturb the spherical contour. Nuclei in which this arrangement exists appear on a cursory examination to contain one large nucleolus and nothing else but nuclear sap. This is the characteristic appearance of the oocyte nucleus in the resting stage.

We have noted that the nucleolus, which is at first vesicular and has little affinity for stain, soon becomes vacuolated and stains more deeply. As it increases in size the vacuoles increase rapidly in number and the affinity for stain becomes more and more marked, till, when the resting stage is reached, the whole surface is covered with vacuoles and the nucleolus stains as deeply as chromatin. Though the nucleolus has so great an affinity for chromatin stains I do not consider that it is formed of chromatin. I regard it rather as a composite structure, consisting of a plasmosome in which chromatin, or some similar basiphil substance, is present. This is indicated by the fact that when stained with eosin the nucleolus is stained bright red, whilst the rest of the ovary is hardly perceptibly tinted by it, but when stained with haematoxylin the red colour is completely masked. These staining properties are confined to a cortical layer, in which the above-mentioned vacuoles lie. This layer surrounds a large central cavity the contents of which appears to be nuclear sap. This structure of the nucleolus is easily seen as it is so large, about 0·015 mm. in diameter, that it may be cut into three or four sections with ease. In such sections the cortical layer appears as a deeply-staining ring surrounding a cavity which contains non-staining material, in which irregular strands of another substance, possibly linin, can be seen (fig. 17).

Though the nucleus normally contains only one large nucleolus this sometimes appears to undergo fragmentation. In the more usual cases of this, one or more small nucleoli

may be seen in the nuclear sap surrounding the large nucleolus, or even inside the central cavity of the latter. The smallest examples of these appear homogeneous, but the larger fragments show a similar vacuolation to that of the large nucleolus, and are evidently produced by the fragmentation of the cortical layer. In one ovary examined all the oocyte nuclei contained numbers of small nucleoli. In some cases, however, the large nucleolus was indicated by a sort of phantom, as if it had given up practically all its substance, but the very small quantity of material remaining still traced its original form. In the cases where the original nucleolus had completely disappeared it is obvious that the fluid which was in its central cavity must have mixed with the surrounding nuclear sap, but there was no indication of two different fluids inside the nucleus. It therefore seems probable that the nucleolus is merely a hollow sphere, with nuclear sap both inside and surrounding it.

The nucleus is surrounded by a thick nuclear membrane which is stained black by iron haematoxylin and frequently shows numerous local thickenings.

When the oocyte has reached the resting stage it has a thick layer of cytoplasm round the nucleus. This usually stains the same as the cytoplasm of the nurse-cells, but sometimes it appears slightly more granular.

Second Period of Growth of the Egg-follicles.

The second period of growth extends from the time when the egg-follicle leaves the resting stage up to the formation of the mature egg. During this period several different processes take place and it will be convenient to consider these separately. These processes may be divided primarily into the nutrition of the egg and the formation of the egg-walls. The complexity of the changes of the oocyte nucleus renders it advisable first to treat with these thoroughly and then to deal with yolk formation and the nutrition of the oocyte in general. The subject of the formation of the egg-walls will be divided into the production of the inner wall, of the outer wall, and of the micropyle apparatus.

I. Branching of the Oocyte Nucleus and
Segregation of Vegetative and Germinal Parts.

During the second period of growth of the egg-follicle of
A. maculipennis the oocyte nucleus undergoes a most
remarkable development. I have not been able to find
a detailed description of a similar development in the case of
any other insect, but, as will be seen later, it is probable that
this particular form of development of the oocyte nucleus
is by no means confined to A. maculipennis.

After the mosquito has fed on blood the first indication of
alteration in form of the oocyte nucleus is observed in the
nuclear membrane. Previously this was spherical in form,
but now it is seen to be somewhat irregular in outline. This
irregularity becomes more and more marked as development
proceeds, till the nuclear membrane is seen to send out a few
blunt processes into the cytoplasm and the cavity enclosed by
the membrane appears somewhat larger than it was previously.

While this has been taking place the nucleolus has also been
altering somewhat in shape. It loses its spherical form, first
becoming ovoid and later slightly flattened in a plane at right
angles to the axis of the egg-follicle, at which stage it begins
to send out blunt processes (fig. 18). The structure, however,
is still the same as in the resting stage, that is, it is vacuolated
and contains a large non-staining central mass.

The chromatin residue commences to separate from the
nucleolus at this stage. Its subsequent history will be dealt
with separately. From this point the more obvious nuclear
changes are confined to the nucleolus and the nuclear mem-
brane, the nucleolus and its products forming by far the greater
part of the bulk of the nucleus.

It has been noticed that both the nucleolus and the nuclear
membrane have begun to send out blunt processes. These
processes rapidly elongate and take on the form of branches,
which in their turn send out secondary branches. The branching
of the nucleolus and the nuclear membrane is intimately
connected, as the nuclear membrane surrounds the nucleolar

branches. The progressive stages of the branching are seen in figs. 1–6, which are reconstructions made from serial sections. In individual sections the branching nature of the nucleus cannot be seen, as only sections of the branches are found and these appear to be fragments of the nucleus, as described by S. R. Christophers (2).

Besides altering in form the nucleolus undergoes an alteration in structure. Shortly after the branching has commenced the vacuoles of the outer crust become indistinct, and the central mass, which up to this point has not taken up stain, now becomes darker and the whole becomes very granular (fig. 19). Later the whole of the products of the nucleolus form a homogeneous granular mass which readily takes up nuclear stains. The branches of the nucleus are entirely formed of this mass and, in the earlier stages at least, are surrounded by the nuclear membrane.

As the branching proceeds the branches become finer and finer, and pass throughout the whole of the rapidly enlarging oocyte. They have, however, a very definite arrangement. It will be seen in figs. 22 and 23 that the main branches occupy approximately a median position between the centre of the egg and the periphery, forming a cup-like structure roughly following the contours of the egg. It must be pointed out, however, that in these two sections the nuclear branches appear much more continuous than is normal, though indications of this arrangement can be seen in all sections of this and later stages. The thickenings of the ring-shaped nuclear mass in fig. 22 are the main branches cut transversely, and the thin portions joining them are smaller lateral branches; these appear to connect the larger branches together, so that probably the nucleus forms a reticular structure from which thin short branches pass towards the centre of the oocyte, while others go towards the periphery. The reconstructions do not show this reticular structure of the nucleus, but this may be accounted for by the fact that only the very finest branches appear to join the main branches together, and I found it impossible to reconstruct the course of such fine branches with

accuracy. They have, therefore, been omitted from the figures of the reconstructions. Thus fig. 6 only shows a large number of more or less longitudinally placed branches, but I consider that these were joined together by a number of much finer branches.

As the branching proceeds the nuclear membrane becomes less conspicuous, but it is easy to see in the earlier stages of nuclear branching. Later it becomes closely surrounded by yolk and evidently lies closely applied to this. The appearance of a membrane in this position can usually be observed, but this by no means proves that a membrane is present. I find that if a crack appears in the yolk-mass, the edges of the crack often appear to be limited by a membrane, and this I believe to be due to the refraction of the transmitted light by the spherical yolk granules. In such cases the apparent membrane always closely follows the contour of the closely-packed yolk granules. In cases of the branching nucleus, therefore, in which the appearance of a membrane can be observed in a position separated from the yolk-mass, I consider that this is actually the nuclear membrane, while, on the other hand, if there appears to be a membrane closely following the limits of the yolk-mass, it cannot be definitely stated that a membrane is, or is not, present. Bearing these considerations in mind, I find that portions at least of the nuclear membrane cover the branches up to a late stage, as when the nurse-cells are breaking down the nuclear membrane can still be seen in places. Whether it is continuous or not at this stage it is impossible to say, but I favour the view that it does not exist over some portions of the branches.

When the nurse-cells are breaking down large deeply-staining globular masses are found in the nuclear branches (fig. 21). These appear to be formed of substance derived from the degenerating nurse-cells. The globular masses are probably absorbed by the nuclear substance as they cannot be observed in later stages.

After the extrusion of the nurse-cell nuclei the main function of the branching nucleus, that of the nutrition of the oocyte,

appears to be completed. It does not immediately degenerate, however, but continues to branch, the branches becoming finer and finer till finally they merge imperceptibly into the cytoplasmic reticulum, when all trace of the nucleus is lost. Occasionally there are still some vestiges of the branches remaining when the egg is laid.

It will be seen later that all this complicated branching of the nucleus may be regarded as a mechanism for the transference of nutritive material to the egg. As has already been noted, this nutritive mechanism is mainly the product of the nucleolus, the nuclear sap and nuclear membrane participating but being only of secondary importance. The nucleolus may therefore be regarded as the vegetative portion of the nucleus. The chromatin residue does not take any part in the nutrition of the egg, but from it the female pronucleus and the polar bodies appear to be produced, so that it is the germinal portion of the nucleus. S. R. Christophers (2) refers to this chromatin residue as the ‘ female pronucleus ’, but as the polar bodies have not yet been separated from it this is obviously a misuse of the term.

When the chromatin residue first begins to leave the side of the nucleolus, it is found to be no longer a deeply-staining mass of chromatin, as only portions of it take up stain readily. Its appearance at this stage varies considerably, but it is usually formed of a non-staining matrix in which a deeply-staining round spot is found, and commonly several other parts take up stain often appearing to be portions of the coiled threads of which it was originally composed (fig. 19). The whole of this is embedded in a mass of lining from which radiating strands pass to various parts of the nucleus.

During the growth of the oocyte the chromatin residue travels progressively farther away from the nucleolus, and as it does so its staining properties decrease. The round spot mentioned above is the last portion to lose its power of taking up chromatin stains, but finally the chromatin residue can only be recognized as a small lightly-staining mass situated a little below the nurse-cells. This is the last stage I have been able

to discover, and it occurs when the egg-follicle is about a third of its full size. It now becomes lost in the yolk-mass from which stains will no longer differentiate it. It does not follow, however, that because it is no longer visible it has therefore ceased to exist as a separate entity.

A little later, after the nurse-cells have been extruded, a small mass of protoplasm which is free from yolk is found situated a little behind the anterior extremity of the egg, that is, approximately in the position in which the chromatin residue disappeared. The central portion of this mass is rather denser than the remainder, and this I regard as the derivative of the chromatin residue. Some eggs were sectionized which had been fixed about an hour after laying. In these a number of minute chromosomes were found situated in the denser central portion of the above-mentioned mass. It would therefore appear that the reconstruction of the chromosomes takes place shortly after the fertilization of the egg, a process which frequently occurs in insect eggs.

As the chromatin residue was derived from the chromatin of the spireme, and as after fertilization the reconstruction of the chromosomes takes place in the anteriorly-placed mass of protoplasm which is free from yolk, it seems a reasonable assumption that this mass contains the derivative of the chromatin residue.

II. Yolk Formation and the Nutrition of the Oocyte.

Shortly after the egg-follicle enters the second period of growth the oocyte commences to enlarge and soon occupies about half of the egg-follicle, the nurse-cells occupying the other half. At this stage yolk begins to make its appearance. First the cytoplasm is seen to contain a number of small globules which do not stain. These enlarge and are then recognized as fine yolk. Immediately after this granules of coarse yolk make their appearance, usually forming a zone midway between the nucleus and the periphery of the oocyte. These granules are very small, but they increase in size as the oocyte

grows, and more small granules or ' young yolk ' appear in the cytoplasm till this is completely filled with yolk. As the oocyte grows, therefore, it is natural that the young yolk should appear at the point where the cytoplasm is increasing most rapidly, that is round the periphery and more particularly at the proximal end of the oocyte. This actually is the case, as will be seen from fig. 27, in which the small granules of young yolk can be plainly seen around the periphery of the oocyte and a much larger mass is visible at its proximal end. Young yolk may also be observed amongst the larger and older granules in the central mass of the oocyte, and no doubt growth is by no means confined to the peripheral portion of the cytoplasm.

At the same time that this coarse yolk is appearing fine yolk is also being laid down in the cytoplasm, the production of the two substances thus taking place simultaneously.

As the oocyte is growing rapidly and large quantities of yolk are being laid down, the question arises as to how the nutrition of the oocyte takes place. The fact that most of the young yolk is laid down in a peripheral position might lead one to suppose that nutritive material passed by diffusion through the follicular epithelium. This probably does take place to some extent, but only in the earliest stages, as later the follicular epithelium begins to secrete the inner wall and then no doubt requires all the nutritive material which passes into it.

The greater part of the nutritive material undoubtedly reaches the egg through the medium of the nurse-cells, and these in their turn must receive it from the ' rosette cells ' as these are the only portion of the epithelium which is not secreting the inner wall. The impushing of the rosette-cells and their close application to the nurse-cells (figs. 36 and 37) may assist in the transference of the nutritive material.

That the nurse-cells are in a state of activity during this period is indicated by the fact that the cytoplasm stains irregularly, more deeply on one side than the other (fig. 27), an appearance which seems to be characteristic of cells which are secreting.

Between the inner side of the nurse-cells and the branching nucleus a mass of cytoplasm is found which stains more deeply than the remaining cytoplasm of the oocyte. This forms a connexion between the nurse-cells and the oocyte nucleus, and I regard it as the path of the nutritive material from the former to the latter (fig. 27).

The branching nature of the nucleus, and the general arrangement of the main branches in a medium position between the centre of the oocyte and its periphery, form an ideal distribution system for carrying nutritive material to all parts of the oocyte.

The path of the nutritive material would therefore appear to be from the surrounding fluid to the rosette-cells, through these into the nurse-cells, which in their turn pass it to the branching nucleus through the medium of the above-mentioned more deeply-staining mass of cytoplasm. The branches carry the fluid to all parts of the oocyte, and the cytoplasm of this uses it in the formation of yolk granules.

When the oocyte is approaching full size the cytoplasm of the nurse-cells begins to disappear (fig. 37) till finally the nuclei are only surrounded by the cell membrane. Simultaneously large globular masses of deeply-staining material appear in the branches of the oocyte nucleus (fig. 21) and obviously have some connexion with the degenerating nurse-cells. These globular bodies are by no means confined to the region of the nucleus near the nurse-cells, but are found in all parts of the main branches, and it is therefore only reasonable to suppose that they have travelled along the branches. This gives considerable support to the view that the branching nucleus is a mechanism for the transference of nutritive material.

It should be noted that the nurse-cells degenerate when the period of nutrition is practically completed, and that in so doing part of their substance is used directly for the nutrition of the oocyte. This is further proof of the nutritive character of the nurse-cells.

As there is no longer any nutritive material for the branching nucleus to carry, it is obvious that if this is its only function

it should now degenerate. This it does, as we have seen, by continuing to branch till the final branches merge into the reticulum of the cytoplasm, when nutrition is completed.

III. Discussion concerning the Oocyte Nucleus and Nutrition of the Oocyte in A. Maculipennis.

I have unfortunately not found it possible to examine the whole of the literature dealing with the nutrition of insect eggs, but, in the literature I have consulted, I have not discovered a case in which the mechanism of nutrition is to my mind as clearly demonstrated as it is in the developing egg-follicle of A. maculipennis. I do not believe, however, that the insect under consideration is unique in having this particular mechanism of nutrition. From several series of sections which I cut of the closely allied insect Theobaldia annulata, I am convinced that the same mechanism is present here. Also Soyer (28) makes a short reference to the nucleus of the developing oocyte of a Staphylinid, and from his description it would appear closely to resemble that of A. maculipennis. He remarks, ' Le noyau, très irrégulier déjà à ses phases les plus jeunes, se ramifie et se déchire en une multitude de franges dans toute l'étendue du vitellus. Cette ramification finit par être poussée si loin qu'on n'a plus sous les yeux qu'une sorte de long filament avec quelques branches latérales, à peine visibles, dont les extrémités se ramifient et se perdent entre les vésicules lécithiques qui emplissent à ce moment la masse ovulaire '. According to Korschelt (13) Stuhlmann has observed the branching of the nucleus throughout almost the entire oocyte of Necrophorus vespillo and of Silpha sp.

Branched nuclei are by no means uncommon, particularly in insects. They are commonly found in nurse-cells, gland-cells, fat body-cells, and the cells of Malpighian tubules, in all of which cases they appear to have some relation to the secretory activities of the cells. Thus in many gland-cells the nucleus is only branched during the period of secretion. In only two

cases have I found references to branched nuclei in cells which
are not obviously secretory in function, but both of these con-
cern embryonal structures which are undergoing rapid growth
and are therefore in a state of great activity. Korschelt (**13**)
cites cases of branched segmentation nuclei, and Seeliger (**25**)
describes the branching of the nuclei in the muscle-bands of
young Oikopleura. In the latter case the branching
reaches an extraordinary high state of development, becoming
finally a complicated reticular network of very fine threads.
Korschelt (**13**) regards the formation of nuclear branches as
a method of increasing the surface of the nuclei to aid secretion.
Thus, speaking of egg-cells, he remarks, ' Die Bildung der
Fortsätze stellt eine Oberflächenvergrösserung des Kernes
dar, vermöge welcher dessen Berührungsfläche mit der
Zellsubstanz erheblich vergrössert wird. In ähnlicher
Weise wurde die Bildung von längeren oder
kürzeren Fortsätzen des Kernes bei secerniren-
den Zellen verschiedener Art beobachtet. Hier
waren die Fortsätze nach demjenigen Theil der
Zelle gerichtet, wo die Secretion stattfand.'

It will thus be seen that the form and position of the nucleus
of the oocyte in A. maculipennis indicates that it is
secretory in function and comparable to the nuclei of secreting
cells. This similarity is further shown by the fact that during
the process of branching the nuclear contents break down and
form a granular mass, a process which normally takes place
in secreting cells during the period of activity. The close rela-
tion of one end of the branching nucleus to the nurse-cells and
the other to the area of maximum activity of the growing
oocyte, i.e. the posterior end, together with the relatively
deeply-staining mass of cytoplasm between the nucleus and the
nurse-cells, renders it difficult to imagine that the branching
nucleus can be other than secretory in function. It is from the
somewhat similar arrangement in other cells that Korschelt (**13**)
draws the conclusion that the nucleus takes an active part in
the nutrition of a cell. Thus he observes, ' Das Aussenden von
Fortsätzen und Annäherung des Kernes an diejenige Seite der

Zelle, von welcher derselben Nährsubstanz zugeführt wird, die Umlagerung des Kernes mit einer von fern her angezogenen Nährmasse,—diese Vorgänge konnten einzig und allein als eine Einflussnahme des Kernes auf die ernährende Thätigkeit der Zelle gedeutet werden.' Also Doncaster (**5**), in his recent work, makes the following assertion : ' The nucleus—in some way controls the metabolic activities of the cell, and its peculiar behaviour in the growing oocyte can only be ascribed to its activities in this connexion.'

Chubb (**4**), on the other hand, denies that the oocyte nucleus takes an active part in yolk formation. Thus he says, ' The actual formation of the yolk spherules must therefore be regarded as an automatic process, which commences as soon as the accumulated materials in the cytoplasm attain the requisite degree of concentration, and which does not entail either increased nutrition of the ovum or increased activity of the nucleus '. The amoeboid movements of the germinal vesicle described by various authors, e.g. Bambeke (**1**), which are considered as an indication of nuclear activity, Chubb regards as probably being artefacts due to fixation. He observed oocyte nuclei in A n t e d o n which were apparently amoeboid, but he shows that these are purely artefacts as ' In the first place the nuclear irregularity shows no spatial relation whatever, either to the other cell structures, to commencing yolk formation or to the position of the nucleus in the cell. In the second place it is only in radial section that the nuclear irregularity presents the appearance of Pseudopodia; in tangential sections these nuclear '' processes '' are found to invariably resolve themselves into a coarse wrinkling of the nuclear membrane. Finally, the artificial nature of the nuclear irregularity is strongly indicated by the variable behaviour of the nucleus with varying fixation.'

It is very probable that this explanation does apply to many cases where amoeboid structure has been described, but it certainly does not apply to the oocyte nucleus of A . m a c u l i - p e n n i s . The high degree of branching of the nucleus in this case could not possibly be regarded as an artefact due to

fixation, and in addition the branching has a definite spatial relation to other cell structures, yolk formation, and the position of the nucleus in the cell. The branched appearance of the nucleus is not confined to any type of section and it is a perfectly constant character in no way dependent on the fixative. Also it is not possible to regard the branches as the result of the pressure of the yolk-laden cytoplasm, so that the only possible explanation is that the oocyte nucleus of A. maculipennis is in a state of great activity during the period of yolk formation.

It has been shown that the oocyte nucleus only commences to branch when the yolk begins to appear, and that when all the yolk has been produced and the nutrition of the oocyte is complete, the branching nucleus breaks down and its substance is absorbed directly by the cytoplasmic portion of the yolk-mass. Immediately before the final disappearance of the branching nucleus this structure rapidly loses its power of taking up stain. This is a further indication of the close similarity existing between the oocyte nucleus of A. maculipennis and the nuclei of secretory cells. Thus Bambeke (1), speaking of glandular cells, points out that after the secretion has lasted for a certain time the power of the nucleus to take up nuclear stains diminishes.

At this point it will be convenient to examine some of the various mechanisms which have for their object the nutrition of the rapidly-growing oocyte. In each case it will be found that the main object of the mechanism is to increase the surface in contact with the cytoplasm of the oocyte, in order to facilitate the passage of nutritive material into the latter.

The activities of the oocyte nucleus in Colymbetes fuscus as described by Will (34) are in many ways not unlike those of the insect under consideration. When the oocyte enters on its period of rapid growth the nuclear membrane becomes irregular and finally many small branches pass into the cytoplasm. Later these become separated from the rest of the nucleus, and are used directly as nutritive material by the cytoplasm. A fresh nuclear membrane develops behind

the separated branch, and the nucleus then produces more branches which in their turn become separated, so that 'der protoplasmatische Leib der Eizelle auf Kosten des Eikernes wächst '. This, however, is not a case of the degeneration of the nucleus, as it continues to increase in size while it is giving up these portions of its substance. Therefore this mechanism of nutrition is practically the same as in A. maculipennis, except that in this case the nucleus continually passes portions of its substance into the growing oocyte as nutritive material instead of merely conducting nutritive fluid to the oocyte.

In Calliphora erythrocephala Lowne (16) describes another manner by which the oocyte receives portions of the nucleus as nutritive material. He says that 'When the egg is enlarged to about two-thirds of its maximum size the granules in the largest nucleus appear to stream out, the nucleus itself shrivels and is ultimately lost, whilst the whole protoplasm of the cell assumes a granular yolk-like appearance, in which the nuclear granules can no longer be distinguished '. The 'largest nucleus ' is evidently the oocyte nucleus, the remainder being those of the nurse-cells. A similar passage of granules from the nucleus has been observed in the oocytes of many insects.

A modification of this process of nutrition has been observed by Gatenby (8) in the oocyte of Apanteles. In this, minute solid chromatoid granules first appear, and later a nuclear membrane appears round each of these. These grow and a livin network appears, and the larger nuclei so formed resemble the true oocyte nucleus to the smallest details. These secondary nuclei disappear when nutrition is complete.

In Rhizotrogus solstitialis Rabes (24) describes a very different mode of nutrition. In this the nutrition of the oocyte is not confined to the nucleus and nurse-cells, the follicular epithelium playing an important rôle. As the oocyte grows the epithelium forms folds which penetrate into the yolk-mass, often as far as the middle of the oocyte, an excellent example of the tendency to increase the surface of contact between the oocyte and secretory structure.

Finally, we may consider the cases in which ' yolk nuclei ' form part of the nutritive mechanism. It is evident that this collective term includes several distinct types of structures, and I will only deal with one of these, the Corpuscles of Balbiani. The origin of this body is obscure in most types which have been examined, but Chubb (**4**) shows very clearly that in the oocyte of A n t e d o n this body arises in the nucleolus as a series of deeply-basophile spherules which are passed into the cytoplasm. These form a mass just outside the nucleus, and eventually they fuse to form the yolk nucleus. McGill (**19**) describes a similar aggregation of granules close to the nucleus in the oocyte of the dragon fly, and this gives rise to the yolk nucleus. Though she has been unable to demonstrate the origin of the granular mass she shows that it is very probably nuclear in origin, and in support of this theory remarks that ' Hennegay (1893) believes that the Corpuscles of Balbiani in Vertebrates are either parts of the nucleolus or the entire nucleolus which passes through the nuclear wall into the cytoplasm '.

Similarly Bambeke (**1**) observes that the ' corps vitellin ' of P h o l c u s p h a l a n g i o i d e s arises close to the germinal vesicle, and he considers that it is nuclear in origin. He shows that this grows into a large and somewhat branched structure which takes an active part in the nutrition of the oocyte. This structure bears a considerable superficial resemblance to the branched nucleus of A . m a c u l i p e n n i s , and a careful consideration of Bambeke's very excellent paper has led me to the conclusion that the resemblance is not merely superficial but that the two structures are both morphologically and physiologically comparable. It should be noted here, however, that Chubb (**4**) considers that the yolk nucleus of A n t e d o n has no connexion with yolk formation though it is almost identical in every respect with the yolk nucleus of P h o l c u s . He gives a perfectly simple physical explanation for the changes undergone by this structure, which he regards as waste material forming a purely passive body.

We have seen that the branched nucleus of A . m a c u l i -

p e n n i s is almost entirely the product of the nucleolus. Now
Bambeke considers that the yolk nucleus of P h o l c u s is nuclear
in origin, and other authors are of the same opinion with regard
to other animals.   Thus Korschelt (**13**) observes, ' Wenn man
sieht, welche complicierte Gestaltung dem aus concentrischen
Schichten gebildeten Dotterkern mancher Spinnen zukommt,
möchte man ihn für einen bedeutungsvollen Bestandtheil des
Kernes halten und ihn gewiss nicht mit dem soeben besprochenen
" Dotterkern " der Amphibien zusammenwerfen.'

In further support of the theory of the nuclear origin of the
Corpuscles of Balbiani, Bambeke remarks : ' Dès que la forme
de bâtonnet a fait place à celle de bourrelet ou de cupule,
la constitution du corps vitellin se montre très semblable, voire
même identique, à celle de la tache germinative. . . . Cette
frappante analogie entre la constitution de ces éléments ne four-
nit-elle pas un argument de plus en faveur de l'origine nucléaire
du corps vitellin ? '

Having shown that the body with which he is dealing is
probably nuclear in origin and is comparable to the nucleolus,
Bambeke proceeds to give his reasons for believing that the
body is a true ' corps vitellin de Balbiani '.   These may be
summarized as follows :

1. Situation near germinal vesicle.
2. Affinity for colours similar to that of the nucleolus.
3. Presence of vacuoles.
4. Constancy of the character.
5. Appearance at commencement of growth.
6. Final degeneration.

All these characters are also true of the branching nucleus
in A . m a c u l i p e n n i s except that in nos. 1 and 2 similarity
of position and character is replaced by identity.   The presence
of vacuoles is only found in the earliest stages of the nucleus,
but this is not actually an important difference from the yolk
nucleus of P h o l c u s , as in the latter the vacuoles disappear
before it degenerates, so that actually this is a further indication
of the similarity existing between the two structures.   Is it

not reasonable, therefore, to consider the yolk nucleus of
Pholcus and the branching nucleus of A. maculipennis
as being homologous structures which only differ in that the
one passes to the outside of the nuclear membrane while the
other remains inside ?

I have already shown that the branching nucleus of the
oocyte in A. maculipennis can only be regarded as
a structure the function of which is to carry nutritive material
to the various parts of the developing oocyte. After an
exhaustive consideration of the various theories as to the func-
tion of the yolk nucleus Bambeke comes to the conclusion that
the only one which can be adopted in the case of Pholcus
' est celle qui considère ce corps comme centre de formation
des éléments nutritifs du vitellus '.

For these reasons I have come to the conclusion already
stated that the branching nucleus of Anopheles
maculipennis and the yolk nucleus of Pholcus
phalangioides are morphologically and physio-
logically comparable. These structures are homologous
with other types of oocyte nuclei and Corpuscles of Balbiani
respectively. It would therefore appear that the Corpuscles of
Balbiani may be considered as portions of the oocyte nucleolus
which have escaped through the nuclear membrane in order
to carry on the nutritive portion of the nuclear functions.

In Pholcus the division of the nucleus into two portions,
one nutritive or vegetative and the other germinal, is only
partial, as the germinal vesicle itself appears amoeboid and
evidently takes part in the nutrition of the oocyte.

In A. maculipennis it has been shown that from an
early stage the nuclear contents are sharply divided into
a vegetative and a germinal portion, the nucleolus and
chromatin residue respectively. During the resting stage there
may be an apparent fusion of the two, but actually they are
only closely applied together, the chromatin residue lying
in an identation of the nucleolus. A close parallel is found in
the ovary of the dragon fly according to McGill (**19**). In this
case the thick spireme of the young oocyte surrounds the

nucleolus, giving rise to a ' double nucleolus '. Later one side
of the nucleolus is formed of chromatin and the other is the
plasmosome.

Gatenby (**8**) shows that in A p a n t e l e s g l o m e r a t u s the
division of the oocyte nucleus into germinal and vegetative
parts takes place in a very different manner. Secondary nuclei
are produced, apparently arising from material which has
escaped from the true oocyte nucleus, and these are found round
the periphery of the oocyte. Then ' some time before the
ovarian oocyte has become ripe the secondary nuclei disappear
by a process of degeneration or chromatolysis '. The secondary
nuclei are considered to influence the production of yolk.
Discussing this subject Gatenby remarks : ' The egg nucleus
of many insects, of which A p a n t e l e s is an example, becomes
partly decentralized ; this is to say, the nucleus, instead of
influencing various processes of oogenesis from afar, sends
pieces of itself into the furthermost regions of the egg, which
carry on part of the vegetative functions at least of the
chromatin of the ordinary nucleus.' This statement applies
equally well to the oocyte nucleus of A . m a c u l i p e n n i s ,
though the pieces sent ' into the furthermost regions of the
egg ' remain attached to the rest of the nucleus.

It has already been shown that, though there is good reason
to believe that the ' chromatin residue ' gives rise to the
segmentation nucleus, there is a period in which no chromatin
matter can be distinguished, and the oocyte of the mosquito
then appears to be without a nucleus. A similar phenomenon
has been encountered in the oocytes of other insects by many
observers. Will (**34**) states that the oocyte nucleus of D y t i s -
c u s becomes a mass of fine granules from a small portion
of which the ' definitive Kern ' is later produced. Lowne (**16**),
speaking of C a l l i p h o r a e r y t h r o c e p h a l a , remarks,
' In the ripe unimpregnated ovum I have entirely failed to
find any nuclei or cellular elements of any kind, and I feel sure
that if any such elements were present they would readily
be distinguished in my sections '. Lubosch (**17**) states that
this disappearance of the staining portions of the oocyte

nucleus for a certain period is the rule rather than the exception in animal eggs, and Doncaster (**5**) makes the following observation on the subject : ' Very commonly the chromosomes . . . disappear, and the chromatin becomes scattered through the nucleus in the form of fine particles, or for a time it may vanish altogether, at least in the sense that it ceases to take up stain.'

The production of the segmentation nucleus at about the period when the egg is laid is the normal occurrence in insect eggs, and it is quickly followed by the polar divisions. Doncaster (**5**) observes that ' in some animals the act of laying seems to be the stimulus and in others the polar division only occurs when a spermatozoon enters the egg ' ; but as in A. maculipennis oviposition and fertilization are simultaneous, it cannot be stated which acts as the stimulus.

In conclusion, the more important points with regard to the oocyte nucleus of A. maculipennis may be summarized as follows :

1. From the earliest stages separate vegetative and germinal portions can be distinguished in the oocyte nucleus.

2. During the second period of growth the nucleus branches throughout the entire oocyte.

3. The branching nucleus, in conjunction with the nurse-cells, takes an active part in the nutrition of the oocyte.

4. The branching nucleus is almost entirely the product of the nucleolus.

5. The branching nucleus is morphologically and physiologically comparable to the Corpuscles of Balbiani of other animals.

6. The germinal portion of the nucleus, the ' chromatin residue ', is the product of the condensation of the spireme threads.

7. The ' chromatin residue ' becomes invisible for a short period and reappears after oviposition as the segmentation nucleus.

IV. Development of the Outer Wall.

The first portion of the outer wall to appear is that which forms the floats. This is secreted between two layers of epithelial cells which come to lie one above the other by a very specialized form of folding of the epithelium.

During the earlier stages of the growth of the follicle the epithelium is of a typically cubical form, but later the cell divisions in two lateral areas become oblique, the obliquity being more marked towards the centre of each area. This process continues with further growth of the follicle (fig. 34) till one much elongated cell lies over the top of several (fig. 35). The underlying cells, however, do not lose their connexion with the tunica propria, but remain attached to it immediately in front of the end of the overlying cell. Finally, it is found that in the two lateral areas there are groups of very much elongated cells which lie almost parallel to the tunica propria. The float is secreted between the outermost of these and the one lying immediately under it (fig. 35). Each corrugation of the float is produced by the secretion of the chorion over the outer surface of one of the much elongated underlying cells.

It will be seen that this overlapping arrangement of the follicle cells is practically a fold of the epithelium. It is not an ordinary epithelial fold, however, as the basement membrane, i. e. the tunica propria, is not disturbed and does not take any part in the folding.

The remainder of the wall makes its appearance shortly after the commencement of the formation of the floats. It is first seen as a simple and very thin membrane lying immediately under the follicular epithelium. Soon local thickenings are found on this membrane (fig. 35). These are the commencement of the processes. The thickenings become larger and grow into the cytoplasm of the epithelial cells. Numbers of such thickenings are formed under each epithelial cell, and the shape of the processes cannot therefore be determined by the form of the secreting cells in the manner which frequently occurs, e. g. in the corrugations of the floats.

G g

The thin membrane of the outer wall does not appear to increase appreciably in thickness, but the processes grow far into the cytoplasm of the epithelial cells till they reach their final size and form. The bosses, in spite of their large size, arise in exactly the same manner as the rest of the processes.

The epithelial layer now undergoes degeneration and becomes separated from the processes till it forms a layer lying over the top of these. Degeneration proceeds till only irregular masses of flattened nuclei can be seen attached to the inner side of the tunica propria (fig. 41), which forms a thin sheath round the whole egg.

V. Development of the Micropyle Apparatus.

The first indication of a special structure being produced for the formation of the micropyle apparatus appears when the egg is about a third of its full size, at the period when the inner wall is beginning to form as a definite layer. At this stage the epithelial cells immediately surrounding the point where the funicle of the secondary follicle joins the primary ovarian follicle become somewhat larger than their neighbours and protrude slightly inwards towards the nurse-cells (fig. 36).

As the egg increases in size this inward protrusion becomes more marked, particularly in the case of the peripheral cells of the group. Finally, the latter are pushed completely inside the epithelial layer and lie between the nurse-cells and the epithelium (fig. 37).

If examined from a surface view these extruded cells are seen to radiate from a common centre, in the form of a rosette, and for that reason I propose to refer to them as rosette-cells (fig. 38).

At this period the cytoplasm of the nurse-cells is seen to be rapidly breaking down and disappearing, and also the contents of the nuclei are degenerating. The chromatin strands lose their definite structure and gradually become a shapeless mass and the nucleoli undergo fragmentation (fig. 37).

The cytoplasm of the rosette-cells becomes very closely

applied to the nurse-cells and gives the appearance of ingesting them.

The nurse-cells, which consist merely of degenerating nuclei invested by the cell membrane, now pass into the epithelium, in which they lie till they become completely degenerated. It will be seen from fig. 39 that they have every appearance of being ingested by an epithelial cell, i.e. a rosette-cell, though I have been unable to demonstrate that they are completely surrounded by the cytoplasm of the rosette-cells. This is not surprising as, owing to the large size of the nurse-cell and the comparatively small size of the rosette-cell, the layer of cytoplasm of the latter surrounding the former would of necessity be exceedingly thin, and would be very difficult to distinguish from the nurse-cell membrane or from the surrounding epithelial cells.

Whether the degenerating nurse-cells are completely ingested by the rosette-cells or not, it is certain that there is a very intimate relation between the two, and the latter invest a considerable portion at least of the former. The degenerating nurse-cell nuclei would appear to form a general food reserve which is used by the rosette-cells while forming the micropyle apparatus.

The large size of the nurse-cells causes the radial arrangement of the rosette-cells to appear distorted, though indications of this arrangement can always be made out.

The micropyle apparatus arises under the rosette-cells at the same time that the rest of the chorion appears. The whole, with the possible exception of the narrow portion of the funnel, is secreted by the rosette-cells, and there is no obvious mechanism to account for the secretion of the thick supporting ring by part of the surface and the thin disk by another.

The bases of the epithelial cells which are surrounded by the rosette-cells pass as fine threads down the funnel, and it is probably these that secrete the funnel, though the bases of the rosette-cells certainly reach the top of the funnel and may pass down it (fig. 39).

As the stopper appears to be a definite portion of the micro-

pyle apparatus it will be convenient to describe its origin
here.

When the rosette-cells are arising from the epithelial cells
and are just protruding slightly towards the nurse-cells, globules
of matter are appearing between the epithelium and the oocyte
over the whole follicle with the exception of this one point.
These globules are the commencement of the inner wall.

If the protruding group of cells is examined carefully it will
be found that there are globules opposite the central cells of
the group (fig. 36). These are the beginning of the stopper and
are exactly the same as those which are giving rise to the inner
wall. The only point in the egg, therefore, where this secretion
is not taking place is a ring corresponding with the rosette-
cells (fig. 37).

As the egg grows this secretion continues till a well-formed
inner wall and a definite mass of similar matter, the stopper,
has appeared.

After the extrusion of the nurse-cells the inner wall narrows
the hole through which they have passed, only leaving sufficient
room for the passage of the funnel, and in so doing passes
over the stopper, so that this now takes up a position imme-
diately beneath the micropyle (fig. 39).

A very similar process of development is described by
Gross (9) for the micropyle apparatus of Xanthogramma
citrofasciata. In this a special group of epithelial cells
is detached from the anterior pole of the egg, and this travels
between the nurse-cells and finally comes to rest immediately
under them. The follicle epithelium grows inward and separates
the group of nurse-cells from the oocyte except in the region
of the detached group of cells. By the time this is completed
the nurse-cells have passed most of their cytoplasm into the
egg-chamber, so that a mass consisting practically only of
nurse-cell nuclei lies over the anterior end of the egg. The
group of cells secretes a ' polsterförmiges Gebilde ', and the rest
of the follicular epithelium secretes the exo- and endo-chorion.
This ' polsterförmiges Gebilde ' comes to lie immediately under
the micropyle apparatus, and is perforated by the micropyle.

It is interesting to note the different manner in which the specialized group of epithelial cells are produced, and the degenerating nurse-cells passed out of the egg chamber in this insect and in A. maculipennis.

The ' polsterförmiges Gebilde ' of Xanthogramma and the ' stopper ' of A. maculipennis are probably homologous, as they are produced in a similar manner by a specialized group of epithelial cells, and they are also similar in appearance and position.   There is one noticeable difference, however : in Xanthogramma the structure is pierced by the micropyle, while in A. maculipennis it appears to be solid, the micropyle terminating immediately above it.

VI. Development of the Inner Wall.

When the egg-follicle has reached about a third of its ultimate size small globules of matter are found between the follicular epithelium and the oocyte.   These are deeply stained by haematoxylin and can be readily distinguished from the yolk granules.   The globules increase in number and size and finally fuse, forming a coat investing the entire oocyte, with the exception of a ring-shaped area under the rosette-cells.

It has already been shown that the inner wall is gelatinous in nature till some time after the egg has been laid, and when in this state rapidly swells in the presence of acids.   It is therefore not surprising that this structure becomes very much distorted during fixation.   In fig. 36 the inner wall is shown as a fibrillar structure, the fibrils stretching across the space between the oocyte and the follicular epithelium.   This is a very common appearance of the inner wall in follicles of about this stage of development, and I regard the fibrils as being produced from globules which adhere to both the oocyte and follicular epithelium and become stretched into threads when these become separated.   In eggs nearing maturity the inner wall appears to be a thick homogeneous layer lying under the follicular epithelium and in it large vacuoles are frequently seen, but the layer does not show any signs of fibrillar structure.

I consider that the substance of the inner wall is no longer in globules but has formed a continuous gelatinous layer. Obviously a fibrillar structure could not be produced from such a layer in the manner described above.

When the egg is freshly laid the inner wall is still a thick gelatinous structure, but after some hours it hardens and in sections is seen to form a thin dark-coloured membrane lying immediately under the outer wall.

As the inner wall appears between the oocyte and the follicular epithelium the question arises as to which of these secretes it. The cytoplasm of the oocyte is already occupied in the production of yolk and the follicular epithelium secretes the outer wall at a later period, so that whichever of these structures form the inner wall is also capable of producing an entirely different substance.

Over the greater part of the egg it is impossible to determine whether the inner wall is secreted by the follicular epithelium or the oocyte; but the stopper, which is merely an isolated portion of the inner wall, is formed between the follicular epithelium and the nurse-cells. The inner wall must therefore be secreted by the follicular epithelium, and after this has been produced the epithelium changes its form of activity and secretes the outer wall.

DEGENERATING EGG-FOLLICLES.

The degeneration of a certain number of egg-follicles seems to be a normal occurrence in the ovary of A. maculipennis. Commonly this degeneration takes place when the follicles are just entering on the second period of growth, but not infrequently at a much earlier stage the primary follicles are found to be represented by a small mass of degenerated cells surrounded by a loose and much-folded tunica propria. The significance of this degeneration is not clear. I have been unable to detect the presence of any bacteria or other organisms, and the fact that degenerating follicles are almost invariably to be found in small numbers in ovaries, but that all, or even

a large part, of the follicles of an ovary have never been found affected, suggests that the phenomenon should be considered as one of atrophy or auto-digestion rather than as a disease. When an ovary is developing the follicles are very crowded and are obviously under compression, and it is probable, therefore, that the removal of several of the follicles from the more crowded parts would benefit the remainder. This may account for the degenerating follicles, but there is nothing but the above consideration to support the theory.

Fig. 33 shows part of the degenerating epithelium of a follicle which has just commenced to produce yolk. It will be noticed that the appearance of degeneration is confined to the epithelium. This is normally the case, and it is only after the epithelium has almost broken down that the central mass of cells degenerates. Each epithelial cell produces one or more large globular masses inside the inwardly-directed portion of its cytoplasm, so that it closely resembles a goblet cell. The masses are very variable in appearance as they stain very irregularly. They are commonly very granular but are otherwise structureless. The thin protoplasmic investment of the globules soon breaks down, so that the globules form a mass which penetrates amongst the nurse-cells.

The mass of cells and globules appears gradually to enter into solution, as it decreases in size till nothing but a few degenerating nuclei and a very loose tunica propria remain to indicate the position of the original follicle (fig. 24).

PRESENCE OF SPOROZOA AND BACTERIA IN EGG-FOLLICLES.

As has already been observed by S. R. Christophers (2), the yolk of a mosquito egg is frequently entirely displaced by a mass of sporozoa. These appear as transparent spherical cysts 0·005 mm. in diameter, approximating in size to the coarse yolk granules, in which eight small bodies which take up stain are found (fig. 10). In sections this number is not constant, but there are never more, and the reduced number is probably due to the removal of part of the cyst. This is the

only stage of the organism which I have observed and, though a number of insects were found affected, the cysts were only observed in mature oocytes.

The nurse-cells of the ovary of one insect were found to be heavily infected with diplococci. The follicles were nearly fully developed, and I could observe no harmful effect of the bacteria. The infection appeared to be entirely confined to the nurse-cells.

SUMMARY.

1. The period at which the ovaries of A. maculipennis commence to develop depends on the season and locality. Normally this is from about the middle of March to the beginning of April.

2. A meal of blood appears to be necessary for the production of eggs.

3. One meal of blood is sufficient to cause eggs to be produced. After the lapse of a day the large blood-mass in the stomach shows two zones : a posterior partially-digested portion and an anterior portion of apparently fresh blood. This appearance has sometimes been taken as evidence that more than one meal of blood has been consumed.

4. The eggs are fully developed six days after the insect has fed on blood.

5. In the case of two insects which were observed at the time of oviposition the eggs were laid immediately after dark.

6. The muscle-bands of the ovarian sheaths are striped ; not unstriped as is usual in insects.

7. A large number of vacuolated cells are found in the ovary. The nature of these is not clear, but they appear to have some relation to the tracheal system.

8. The chorion of the egg is highly specialized to retain air round the egg, and the buoyancy of the egg is entirely due to the entrapped air.

9. The floats are produced by a very specialized form of folding of the follicular epithelium.

10. The micropyle apparatus is produced by specialized cells of the epithelium, the ' rosette-cells '.

11. Immediately below the micropyle is a specialized portion of the inner wall, the ' stopper '.

12. The inner and outer walls of the egg, though formed of entirely different substances, are both secreted by the follicular epithelium.

13. The inner wall is first gelatinous in nature and transparent ; but, after the egg is laid, becomes brittle and dark in colour, causing the egg to appear black.   This change in character only takes place when the eggs are laid on water.

14. The mature egg contains two distinct kinds of yolk, one of large granules which are proteid in nature, and the other of small granules the nature of which I have been unable to determine.

15. There are two distinct periods of growth of the egg-follicles, the first culminating in the ' resting stage ' and the second only commencing after the mosquito has had a meal of blood.

16. Each egg-follicle consists of a follicular epithelium surrounding seven nurse-cells and an oocyte.   These appear to be the product of a single oogonium.

17. The cells of the follicular epithelium multiply by mitotic division during the whole of the first period of growth.   In the second period, though the follicular epithelium increases greatly in area, this is due purely to the increase in size of the individual cells.

18. From the earliest stages separate vegetative and germinal portions can be distinguished in the oocyte nucleus.

19. During the second period of growth the oocyte nucleus branches throughout the entire oocyte.

20. The branching nucleus, in conjunction with the nurse-cells, takes an active part in the nutrition of the oocyte.

21. The branching of the nucleus may be regarded as a mechanism for the purpose of increasing the surface.

22. I have observed a similar method of branching of the oocyte nucleus in Theobaldia annulata, and it

probably also exists in Necrophorus vespillo and
Silpha sp.

23. The branching nucleus is almost entirely the product
of the nucleolus.

24. The branching nucleus is morphologically and physiologi-
cally comparable to the Corpuscles of Balbiani of other animals.

25. The germinal portion of the nucleus, the ' chromatin
residue ', is the product of the condensation of the spireme
threads.

26. The ' chromatin residue ' becomes invisible for a short
period and reappears after oviposition as the segmentation
nucleus.

27. The chromatin of the active nurse-cells consists of
minute bars situated on a much convoluted band of linin.

28. Degeneration of a certain number of egg-follicles is
normal during the development of the ovary.

29. Sporozoa are frequently found in the eggs, often .com-
pletely replacing the whole of the yolk.

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EXPLANATION OF PLATES.

REFERENCE LETTERS.

$b.$ = ' Bosses '. $c.c.$ = Central cavity of nucleolus. $c.l.$ = Cortical layer of nucleolus. $c.r.$ = ' Chromatin residue '. $c.y.$ = Coarse yolk granules. $D.$ = Dorsal surface. $d.$ = Disk of micropyle apparatus. $d.p.$ = Dorsal processes. $f.$ = Float. $f.e.$ = Follicular epithelium. $f.m.$ = Follicular tube membrane. $fu.$ = Funicle. $f.y.$ = Fine yolk granules. $g.$ = Gynaecophoric canal. $i.m.$ = Investing membrane. $i.w.$ = Inner wall. $m.$ = Mucous gland. $m.a.$ = Micropyle apparatus. $m.b.$ = Muscle bands. $n.$ = Nurse-cells. $n.m.$ = Nuclear membrane. $o.c.$ = Oocyte cytoplasm. $o.n.$ = Oocyte nucleus. $o.t.$ = Ovarian tube. $o.w.$ = Outer wall or chorion. $p.f.$ = Primary follicle. $r.c.$ = Rosette-cells. $s.$ = Spermatheca. $s.f.$ = Secondary follicles. $s.p.$ = Suspensory filament. $s.r.$ = Supporting ring of micropyle apparatus. $st.$ = ' Stopper '. $t.$ = Tracheae. $t.p.$ = Tunica propria. $V.$ = Ventral surface. $v.c.$ = Vacuolated cells. $v.p.$ = Ventral processes. $y.$ = Yolk.

Figs. 1–6.—Reconstructions of progressive stages of branching of oocyte nucleus. Same scale. N o t e.—Branches overlie, and do not enter nurse-cells.

Fig. 1.—Resting stage.

Fig. 2.—Nucleus becoming irregular.

Fig. 3.—Commencement of branching. Nucleus still vacuolated.

Fig. 4.—Later stage of branching, vacuoles have disappeared.

Fig. 5.—Branching nucleus in half-developed follicle.

Fig. 6.—Branching nucleus in full-sized oocyte, after extrusion of nurse-cells.

Fig. 7.—Adult female genital organs. Ovaries in resting stage.

Fig. 8.—Ovary containing full-sized oocytes.

Fig. 9.—Egg after deposition. Anterior end at top of figure. A. Dorsal view. B. Lateral view. C. Median transverse section.

Fig. 10.—Sporozoa from yolk-mass.

Fig. 11.—Section of yolk-mass.

Figs. 12–21.—Progressive stages of oocyte nucleus. 12–16 scale of 15. 17–21 scale of 17.

Fig. 12.—' Bouquet stage '.

Fig. 13.—Spireme surrounding nucleolus.

Fig. 14.—Nucleolus becoming free from spireme.

Fig. 15.—Nucleolus becoming vacuolated.

Fig. 16.—Spireme condensing.

Fig. 17.—Resting stage. Spireme condensing to form chromatin residue.

Fig. 18.—Commencement of second period of growth. Chromatin residue losing staining properties.

Fig. 19.—Slightly later stage. Chromatin residue separating from nucleolus which has practically lost vacuolated structure.

Fig. 20.—Portion of nuclear branch in half-developed follicle.

Fig. 21.—Portion of nuclear branch containing globular masses at period when nurse-cells are breaking down.

Fig. 22.—Transverse section of half-developed follicle, showing ring-like formation of branching nucleus.

Fig. 23.—Longitudinal section of follicle at same stage, showing position of nuclear branches.

Fig. 24.—Longitudinal section of secondary follicle and end chamber. Folded tunica propria left by degenerated primary follicle.

Fig. 25.—Longitudinal section of secondary follicle. One nurse-cell nucleus contains spireme.

Fig. 26.—Longitudinal section of follicle in resting stage.

Fig. 27.—Longitudinal section of follicle at beginning of second period of growth, showing denser cytoplasm between nurse-cells and oocyte nucleus, commencement of inner wall and yolk production.

Fig. 28.—Musculature of investing membrane.

Fig. 29.—Muscles of investing membrane and follicular tube membrane, showing transition to circular and longitudinal muscles of oviduct.

Fig. 30.—Nurse-cell nucleus in resting stage. Tangential section.

Fig. 31.—Transverse section of end chamber containing mitotic figure.

Fig. 32.—Mitotic division in follicular epithelium cells during first period of growth.

Fig. 33.—Degenerating follicular epithelium.

Fig. 34.—Early stage of epithelial folding for float formation. Somewhat distorted section chosen as it clearly shows limits of epithelial cells.

Fig. 35.—Later stage of folding. Float and outer wall with commencement of processes secreted.

Fig. 36.—Commencement of differentiation of rosette-cells and production of ' stopper '. Longitudinal section.

Fig. 37.—Later stage of same. Nurse-cells degenerating. Longitudinal section.

Fig. 38.—Transverse section of rosette-cells at same stage as 37.

Fig. 39.—Longitudinal section. Degenerating nurse-cell nuclei shown partially surrounded by rosette-cells.

Fig. 40.—Surface view of micropyle apparatus.

Fig. 41.—Longitudinal section of anterior end of egg, showing section of micropyle apparatus and position of ' stopper '.